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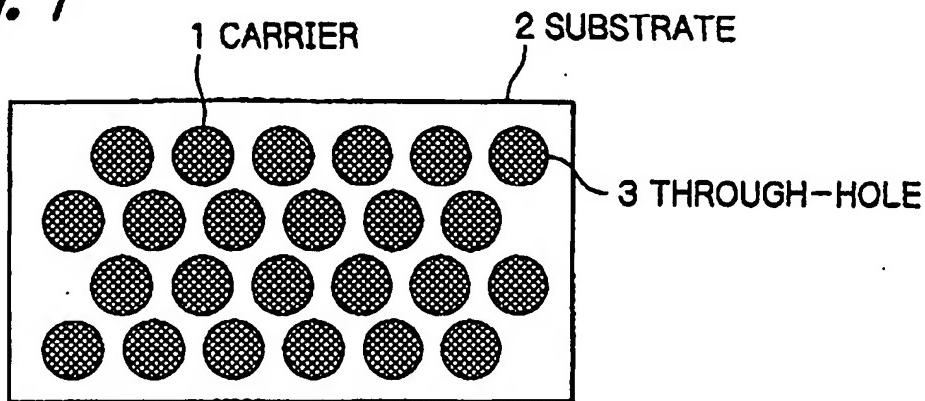
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## (54) Reactive probe chip and reactive detection system

(57) A reaction probe chip comprising a substrate having a plurality of discrete, regularly arranged through-holes; and a carrier filled into and held in the through-holes, the carrier having probe molecules fixed thereto such that the probe molecules are different according to the through-holes. The carrier having the probe molecules fixed thereto is preferably a porous membrane, a nonwoven fabric, or porous glass entangled with or bound to the porous membrane or nonwo-

ven fabric. A reaction product detection system flows a sample, including fluorescence labeled DNA to be detected, simultaneously and slowly through a plurality of discrete through-holes regularly arranged in a substrate, thereby binding an analyte to probe molecules, and detects the analyte by a fluorescence detector. The reaction probe chip and the detection system based on a convenient method for preparing DNA Chip can be used in various diagnoses of physiological functions, including DNA polymorphism.

Fig. 1



**Description**

**[0001]** This invention relates to a reaction probe chip, which is used for diagnosis of genes and physiological functions and which enables recognition of many functional molecules, and a detection system for detecting a reaction product obtained by the reaction probe chip.

**Description of the Related Art**

**[0002]** Detection of polymorphism due to variation of a gene, especially variation of one base (sequence), is effective for diagnosis of a disease ascribed to mutation or the like, e.g., cancer. Its detection is also necessary for guidelines on drug response and adverse reactions, and contributes to analysis of genes related to causes of multiple factor diseases and contributes to predictive medical care. The use of a so-called DNA chip is known to be effective for this detection.

**[0003]** A hitherto utilized DNA chip having short DNA strands fixed thereto, so-called Gene Chip of Affymetrix, usually comprises 10,000 or more oligo-DNA fragments (DNA probes) fabricated on a silicon or glass substrate 1 cm square by photolithography technology. When a DNA sample, for example, labeled with fluorescence is flowed on this DNA chip, DNA fragments having sequences complementary to the probes on the DNA chip bind to the probes. Only the bound portions can be distinguished by fluorescence, and the particular sequences of the DNA fragments in the DNA sample can be recognized and quantitatively determined. This method has already been shown to be capable of detecting mutation of an oncogene or detecting gene polymorphism.

**[0004]** A microarray having cDNA's arranged on a slide glass is also used.

**[0005]** The earlier technologies, however, have posed some problems.

**[0006]** For DNA Chip to be produced using photolithography, for example, at least four photomasks are needed for synthesis in one stage, and a cycle of photolithography, coupling and washing has to be repeated four times. Since this procedure is repeated for the necessary strand length, a high cost is involved. In changing patterns, the photomasks need to be changed accordingly. DNA Chip's of various designs, which flexibly meet the needs, have not been available.

**[0007]** DNA Microarray Chip, spotted with a solution of synthetic oligonucleotides at a high density, has been proposed as an alternative method. This type of chip has to undergo a complicated procedure which comprises introducing a modification group in succession to oligonucleotide synthesis, cutting off the modified oligonucleotides from the carrier, followed by detachment and purification, to obtain oligonucleotides, and reacting the oligonucleotides with functional groups introduced onto fixation glass. Thus, this chip is costly, like DNA Chip relying on photolithography.

**[0008]** During detection with these reaction chips, hy-

bridization is localized to lead to a loss in quantitative determinability, and a device dedicated to hybridization should be used and put to a long-term reaction. Thus, detection of various pieces of genetic information, including one for the purpose of bone marrow transplantation, by use of the reaction chips is laborious and time-consuming, and incurs a lot of expenses.

**[0009]** The object of the present invention is to establish a more convenient method for preparing DNA Chip, and to provide a reaction detecting chip and a detection system which can be used in various diagnoses of physiological functions, including DNA polymorphism.

**[0010]** To attain the foregoing object, the present inventors conducted various studies on materials for and shapes of reaction probe chips. Through such studies, they attempted to realize a reaction probe chip, which has on the surface a high integration degree comparable to that obtained by the aforementioned photolithography facilities, without using photolithographic technologies requiring a lengthy, complicated reaction process, posing difficulty in flexibly attaining different objects, and involving huge costs, and without employing a device dedicated to hybridization.

**[0011]** Based on these studies, the inventors found that the above-described problems could be solved by filling and holding carriers into a plurality of through-holes regularly arranged in a substrate, the carriers having different probe molecules fixed for the respective holes, and then introducing a sample into the carrier-filled holes. This finding led them to accomplish the present invention.

**[0012]** The present invention solves the above problems by the following means:

**35** (1) A reaction probe chip comprising:

a substrate having a plurality of discrete, regularly arranged through-holes; and  
40 a carrier filled into and held in the through-holes, the carrier having probe molecules fixed thereto such that the probe molecules are different according to the through-holes.

If necessary, the probe molecules fixed to through-holes may be the same according to two or more through-holes in a chip. Alternatively, two or more different probe molecules may be fixed in one through-hole.

**50** (2) The reaction probe chip of (1), wherein  
the carrier having the probe molecules fixed thereto is a porous membrane or a nonwoven fabric, and  
the porous membrane or the nonwoven fabric is pasted to the substrate so as to close the through-holes.

**55** (3) The reaction probe chip of (1), wherein  
the carrier having the probe molecules fixed thereto is a powder of porous glass, and

the powder of porous glass is entangled with or bound to a porous membrane or a nonwoven fabric pasted to the substrate so as to close the through-holes.

(4) The reaction probe chip of any one of (1) to (3), wherein

the probe molecules are DNA's, RNA's or PNA's and fragments thereof, oligonucleotides having arbitrary base sequences, antigens, antibodies or epitopes, and enzymes, proteins or functional site polypeptide chains thereof.

(5) A reaction product detection system adapted to flow a sample simultaneously and slowly through a plurality of discrete through-holes regularly arranged in a substrate, the sample including fluorescence labeled DNA to be detected, thereby binding an analyte to probe molecules fixed in the through-holes, and

detect the analyte by a fluorescence detector.

[0013] According to the present invention, there can be easily provided a reaction detection chip which has reactive substances integrated on its surface, the reactive substances including proteins having arbitrary configurations or oligonucleotides having arbitrary base sequences. The detection chip can be provided without the need for special equipment such as photolithography equipment.

[0014] Moreover, the reaction time can be shortened dramatically, the reproducibility of the reaction can be improved, and the total throughput can be increased. Hence, the preparation of a reaction detection chip for DNA or the like, which meets individuals' needs, becomes possible, contributing to made-to-order medical care.

[0015] The present invention will become more fully understood from the detailed description given hereinbelow and the accompanying drawings which are given by way of illustration only, and thus are not limitative of the present invention, and wherein:

FIG. 1 is a plan view of a reaction probe chip according to the present invention;

FIGS. 2(a) to 2(d) are partial sectional views showing the state of disposition of various carriers in through-holes of a substrate in the reaction probe chip of the present invention, the carriers in FIGS. 2(a) to 2(d) being a porous substance, a porous membrane, a nonwoven fabric, and a porous glass powder fixed to the membrane or the like, respectively;

FIGS. 3(e) to 3(g) are enlarged partial sectional views illustrating the porous substance, the nonwoven fabric, and the porous glass powder fixed to the nonwoven fabric, respectively;

FIGS. 4(h) and 4(i) are enlarged partial cross-sectional views of the carriers having probe molecules fixed thereto, the carriers being the porous sub-

stance in FIG. 4(h) and the nonwoven fabric in FIG. 4(i);

FIG. 5 shows, in cross section, the reaction probe chip of the present invention having the carrier held in the holes, and a stack of the reaction probe chips; FIGS. 6(j) to 6(l) are sectional explanatory drawings of the reaction probe chip of the present invention fixed in a reaction cell, FIG. 6(j) illustrating the fixed state of the reaction chip in the reaction cell, FIG. 6(k) illustrating the initial state of feeding and suction of a fluorescence labeled sample, and FIG. 6(l) illustrating the distributed state of hybridized spots in the reaction chip;

FIG. 7 is a sectional view illustrating the principle of fluorescence detection showing fluorescence emission from the hybridized spots in the reaction probe chip of the present invention when exposed to ultraviolet radiation;

FIG. 8 is a plan view showing the distributed state of spots upon hybridization of the reaction probe chip of the present invention;

FIG. 9 is a conceptual view of a fluorescence detection system using the reaction probe chip of the present invention; and

FIG. 10 is a schematic drawing of a detection system in which the conceptual view of the fluorescence detection system of the present invention in FIG. 9 is expressed as devices.

[0016] Embodiments of the present invention will be described in more detail with reference to the accompanying drawings.

[0017] FIG. 1 is an explanatory drawing showing the relationship between a substrate constituting the reaction probe chip of the present invention and a carrier having probe molecules fixed thereto, the substrate having a plurality of discrete through-holes regularly arranged therein. FIG. 1 illustrates, in a plan view, the arranged state of plural through-holes 3 in a substrate 2 which are filled with and hold a carrier 1.

[0018] FIGS. 2(a) to 2(d) are partial sectional views showing the state of disposition of various carriers 1 in the through-hole 3 of the substrate 2 in the reaction probe chip shown in FIG. 1. The carriers are liquid permeable, and materials having such properties are collectively called "porous medium or media". Various materials can be used as the porous media. FIG. 2(a) shows a state in which a porous substance 1a as the carrier 1 is filled to the full and held in the through-hole 3. FIG. 2(b) shows a state in which the porous substance 1a is filled in a lower half of the through-hole 3. FIG. 2(c) shows a state in which a porous membrane 1b or a nonwoven fabric 1c, as the carrier 1, is pasted to the lower surface of the through-hole 3. FIG. 2(d) shows a state in which a fine powder of porous glass 1d is further fixed, as a carrier, to the membrane 1b or nonwoven fabric 1c. The porous membrane 1b has liquid permeable micropores, and includes a microporous film.

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[0019] FIGS. 3(e) to 3(g) are enlarged sectional views showing the microstructures of the porous media used in FIGS. 1 and 2(a) to 2(d). In these drawings, black portions represent the solid portions of the porous medium, while white portions represent the porous portions of the porous medium. FIG. 3(e) is an enlarged sectional view of the porous substance 1a as the carrier 1. FIG. 3(f) is an enlarged sectional view of the nonwoven fabric as the carrier 1. FIG. 3(g) is an enlarged sectional view showing the entangled binding of the fine porous glass powder 1d fixed to the fibers of the nonwoven fabric 1c.

[0020] FIGS. 4(h) and 4(i) are enlarged sectional views showing the microstructures of the carriers 1 having probe molecules 4 fixed thereto. FIG. 4(h) illustrates the state of the probe molecules 4 fixed in the porous substance 1a (shaded portions), FIG. 4(i) illustrates the state of the probe molecules 4 fixed to the surfaces of the fibers (shaded portions) of the nonwoven fabric 1c.

[0021] FIG. 5 is a sectional view of the reaction probe chip 5 in which the carrier 1 having different probe molecules 4, 4, and so on fixed thereto is filled and held in a plurality of the through-holes 3, 3, etc. regularly arranged in the substrate 2, along with a sectional view showing a multi-layer stack of the reaction probe chips 5 for simultaneously presenting many of the reaction probe chips 5, 5 ... having the same characteristics.

[0022] FIGS. 6(j) to 6(l) are sectional views for explaining the status of detection of a sample with the use of the reaction probe chip 5 fixed in a reaction cell 6. FIG. 6(j) is a sectional view showing a state of fixing, in which the reference numeral 7 denotes a sample inlet, 8 denotes a sample suction port, and the other numerals denote portions having the same functions as described earlier. FIG. 6(k) is a sectional view showing a state in which a fluorescence labeled sample 9 begins to be fed and sucked. FIG. 6(l) is a sectional view of hybridized spots 10, 10 upon binding to several types of specific probe molecules 4.

[0023] FIGS. 7 and 8 are views illustrating the principle of detection of the hybridized spots 10, 10 by a fluorescence detector. FIG. 7 is a sectional view for explaining a state in which the reaction probe chip 5 having the hybridized spots 10, 10 shown in FIG. 6(l) is irradiated with ultraviolet (UV) radiation 11, and only the spots 10, 10 emit fluorescence 12. FIG. 8 is a plan view showing the distributed state of the through-holes 3 having the hybridized spots 10, 10 among the plural through-holes 3 regularly arranged in the substrate 2. FIG. 8 also shows the locations of emission of the fluorescence 12 at the spots, 10, 10 ... in the plural through-holes 3 of the substrate 2.

[0024] FIG. 9 is a conceptual view of a fluorescence detection system using the reaction probe chip of the present invention. In a detection portion 14, ultraviolet radiation from an excitation light source 15 is directed at the reaction probe chip, fluorescence emitted is subjected to fluorescence detection 16, and data on the detected fluorescence is transferred to a data processing

portion 17 for processing of the data.

[0025] FIG. 10 expresses the conceptual view of FIG. 9 schematically as devices. This drawing shows a detection system in which hybridized spots obtained in a hybridization device 18 are observed for fluorescence by a fluorescence observation device 19 composed of a light source portion 20, a module accommodation portion 21, and an observation unit 22, and data obtained are processed by the data processing portion 17.

[0026] Next, the materials for and dimensions of the substrate, carrier, etc. described in the drawings are explained.

[0027] The reaction probe chip of the present invention can be prepared by a process comprising the steps of:

providing a substrate;  
opening a regular array of discrete through-holes in the substrate that connect at least two of its surfaces;  
fixing a carrier having a reactive surface, such as a powder of porous glass, a porous membrane or non-woven fabric, to at least one of the through-holes, which carrier bears on its surface a reacting substance such as DNA, RNA or PNA, antigen, antibody or epitope thereof, enzyme, protein or a functional fragment thereof.

[0028] Alternatively, the reactive substance may be carried on the surface of the carrier after fixing the latter to at least one of the through-holes.

[0029] The substrate may be a material unchanged and stable to the detection system, and needs to have surface characteristics suitable for fixing of the carrier.

[0030] The preferred substrate is a glass substrate such as quartz glass or boro-silicate glass, or an inorganic substrate such as a silicon wafer. However, an organic substrate, such as a polyester film or a polyethylene film, can be used, if a method for bonding it to the carrier can be worked out. Suitable surface treatment can be applied to the surface of the substrate in order to adjust, for example, compatibility with a carrier binder.

[0031] In terms of its shape, the substrate is particularly preferably a flat plate, such as a film or a sheet. If the substrate is in the plate form, the thickness or size of the substrate is not restricted. The thickness of the substrate is determined, as desired, in consideration of shape stability required of the substrate. The size of the substrate is determined, as desired, in consideration of, for example, the number of the through-holes provided in the surface of the substrate.

[0032] The dimension of the plural through-holes regularly arranged in the substrate is not restricted, but is preferably 0.5 to 2 mm, particularly preferably about 1 mm.

[0033] The carrier is a material for bearing a reactive substance (probe molecule), such as DNA, RNA or PNA, antigen, antibody or an epitope thereof, enzyme,

protein or a functional site polypeptide chain thereof. Preferred examples of the carrier are porous materials, such as a powder of porous glass, a porous membrane, and a nonwoven fabric. The shapes of the pores may be any shapes, as long as the porous material has a porous structure. Suitable surface treatment is preferably applied to the surface of the carrier in order to adjust, for example, compatibility with the reactive substance. The carrier in the through-hole should have a structure in which when a liquid is flowed during carriage of the reactive substance or during reaction for detection, the liquid can be flowed through the through-hole from top to bottom.

[0033] That is, the porous material, as the site of reaction, may be a material which fixes or grows the reaction probe molecule. There is no restriction on the type of the material, but porous glass powder or a glass fiber filter paper (nonwoven fabric) is preferred.

[0034] The method of carrying the reactive substance (probe molecule) onto the carrier need not be specified. However, any means usable for fixing can be used, such as a method which comprises fixing an amino group to the surface of the carrier, and then fixing a polypeptide chain with the use of glutaraldehyde. The reactive substance of a particular size need not be fixed and carried, but it is permissible to synthesize the reactive substance on the carrier as in the synthesis of oligonucleotide or oligopeptide, and use it unchanged.

[0035] The size and shape of the carrier can be selected arbitrarily. In view of the fact that the carrier bearing many various reactive substances is fixed onto the substrate, however, the preferred carrier is a powder with 1 to 100 microns, particularly preferably 3 to 20 microns. This is because a larger particle size is preferred in terms of the work efficiency of the process for carrying the reactive substance, but a smaller particle size is preferred when fixing the carrier after carriage of the reactive substance. The fixation of the carrier to the substrate is performed by dispersing the carrier in a solvent, such as water, together with a cellulose-based adhesive, such as cellulose nitrate, and arranging or printing the dispersion onto the substrate by means of a dispenser, spotter or the like.

[0036] The pore size of the porous glass powder or membrane is preferably 0.1 to 0.5 µm, and the fine gaps of the nonwoven fabric or filter paper are preferably several micrometers or less. Too small a pore size makes it difficult to filter the fluorescence labeled sample, so that a pore size of 0.1 µm or more is necessary.

[0037] The "reactivity" in the "reactive probe" described in the present invention refers not only to the properties of changing a chemical structure or the like ascribed to ionic bond or covalent bond by a chemical reaction, but also to the properties of bringing about binding to other substance by other mode, such as van der Waals force, hydrogen bond, coordinate bond, chemical adsorption, or physical adsorption. Examples of such a reactive probe are, but of course not restricted

to, enzymes, antigens, DNA fragments, antibodies, epitopes, and proteins.

5 [0038] If an oligonucleotide, a DNA fragment synthesized on the carrier, is used as the reactive probe, for example, its hybridization with a DNA sample to be detected can result in the detection of DNA having a particular sequence.

10 [0039] Preparation processes for the reaction probe chip of the present invention, and a detection system using it will be described.

[0040] The reaction probe chip of the present invention has a structure in which the substrate is perforated with the through-holes and the reaction probes are fixed there (FIGS. 1 and 2(a) to 2(d)). At the site of reaction, such as the porous medium or nonwoven fabric, the probe molecules are fixed as shown in FIGS. 4(h) and 4(i).

15 [0041] The shape of the reaction site in the through-hole may include some cases, as shown in FIGS. 2(a) to 2(d). In any of these cases, the through-hole should be enough large to allow passage of a gene or the like to be analyzed, and should be designed to hold a reactive probe molecule.

20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140 145 150 155 160 165 170 175 180 185 190 195 200 205 210 215 220 225 230 235 240 245 250 255 260 265 270 275 280 285 290 295 300 305 310 315 320 325 330 335 340 345 350 355 360 365 370 375 380 385 390 395 400 405 410 415 420 425 430 435 440 445 450 455 460 465 470 475 480 485 490 495 500 505 510 515 520 525 530 535 540 545 550 555 560 565 570 575 580 585 590 595 600 605 610 615 620 625 630 635 640 645 650 655 660 665 670 675 680 685 690 695 700 705 710 715 720 725 730 735 740 745 750 755 760 765 770 775 780 785 790 795 800 805 810 815 820 825 830 835 840 845 850 855 860 865 870 875 880 885 890 895 900 905 910 915 920 925 930 935 940 945 950 955 960 965 970 975 980 985 990 995 1000 1005 1010 1015 1020 1025 1030 1035 1040 1045 1050 1055 1060 1065 1070 1075 1080 1085 1090 1095 1100 1105 1110 1115 1120 1125 1130 1135 1140 1145 1150 1155 1160 1165 1170 1175 1180 1185 1190 1195 1200 1205 1210 1215 1220 1225 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3230 3235 3240 3245 3250 3255 3260 3265 3270 3275 3280 3285 3290 3295 3300 3305 3310 3315 3320 3325 3330 3335 3340 3345 3350 3355 3360 3365 3370 3375 3380 3385 3390 3395 3400 3405 3410 3415 3420 3425 3430 3435 3440 3445 3450 3455 3460 3465 3470 3475 3480 3485 3490 3495 3500 3505 3510 3515 3520 3525 3530 3535 3540 3545 3550 3555 3560 3565 3570 3575 3580 3585 3590 3595 3600 3605 3610 3615 3620 3625 3630 3635 3640 3645 3650 3655 3660 3665 3670 3675 3680 3685 3690 3695 3700 3705 3710 3715 3720 3725 3730 3735 3740 3745 3750 3755 3760 3765 3770 3775 3780 3785 3790 3795 3800 3805 3810 3815 3820 3825 3830 3835 3840 3845 3850 3855 3860 3865 3870 3875 3880 3885 3890 3895 3900 3905 3910 3915 3920 3925 3930 3935 3940 3945 3950 3955 3960 3965 3970 3975 3980 3985 3990 3995 4000 4005 4010 4015 4020 4025 4030 4035 4040 4045 4050 4055 4060 4065 4070 4075 4080 4085 4090 4095 4100 4105 4110 4115 4120 4125 4130 4135 4140 4145 4150 4155 4160 4165 4170 4175 4180 4185 4190 4195 4200 4205 4210 4215 4220 4225 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[0044] The present invention will be described in further detail by reference to the following examples, which in no way limit the invention.

**Example 1:**

[0045] A quartz glass substrate (length 70 mm, width 25 mm, thickness 0.5 mm) having 10 × 5 regularly arranged through-holes of 1 mm in diameter was prepared. A powder of porous glass with a pore size of 100 nm and a particle size of 10 microns was filled into the through-holes, and baked. The upper and lower surfaces of the substrate were smoothed by polishing. The substrate was chemically cleaned, and then surface amminated using a silane coupling agent. Ten of the substrates were superposed, one on another, and different types of cDNA's were fixed to the respective through-holes by the customary method.

[0046] The substrates were placed in a reaction cell of polypropylene, and fluorescence labeled cDNA to be detected was poured into the reaction cell, and reacted. After reaction and washing, the chips were withdrawn from the cell, and analyzed by a fluorescence detector.

**Example 2:**

[0047] A polyethylene terephthalate substrate (length 50 mm, width 30 mm, thickness 0.3 mm) having an arrangement of through-holes of 2 mm in diameter was prepared. A glass fiber filter paper was sandwiched between the substrates, and the composite was heat sealed to prepare a reaction chip substrate. One-hundred of the substrates were superposed, one on another, and reagents were sequentially passed through the respective holes vertically communicating with each other to synthesize different oligonucleotides.

[0048] The substrates were placed in a reaction cell of polypropylene, and fluorescence labeled cDNA to be detected was poured into the reaction cell, and reacted. After reaction and washing, the chips were withdrawn from the cell, and analyzed by a fluorescence detector.

**Example 3:**

[0049] A Pyrex glass substrate (length 80 mm, width 30 mm, thickness 0.5 mm) having an arrangement of through-holes of 0.5 mm in diameter was prepared. A porous membrane comprising regenerated cellulose was pasted to the upper surface of the substrate. Separately, a powder of porous glass with a pore size of 100 nm and a particle diameter of 5 microns was prepared, and various oligonucleotides were synthesized by the customary method. These materials were dipped into the respective holes, and a dilute solution of cellulose nitrate, a cellulose-based adhesive, was added drop-wise to fix the porous glass powder.

[0050] The substrates were placed in a reaction cell of polypropylene, and fluorescence labeled cDNA to be

detected was poured into the reaction cell, and reacted. After reaction and washing, the chip was withdrawn from the cell, and analyzed by a fluorescence detector.

[0051] In all of the Examples, the hybridized spots emitted a strong fluorescence upon exposure to ultraviolet radiation. The sequences of DNA fragments in the DNA sample were elucidated by the fluorescence detector.

[0052] While the present invention has been described in the foregoing fashion, it is to be understood that the invention is not limited thereby, but may be varied in many other ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the appended claims.

**Claims**

- 20 1. A reaction probe chip comprising:  
25     a substrate having a plurality of discrete, regularly arranged through-holes; and  
25     a carrier filled into and held in the through-holes, the carrier having probe molecules fixed thereto such that the probe molecules are different according to the through-holes.
- 30 2. The reaction probe chip of claim 1, wherein  
35     the carrier having the probe molecules fixed thereto is a porous membrane or a nonwoven fabric, and  
35     the porous membrane or the nonwoven fabric is pasted to the substrate so as to close the through-holes.
- 40 3. The reaction probe chip of claim 1, wherein  
45     the carrier having the probe molecules fixed thereto is a powder of porous glass, and  
45     the powder of porous glass is entangled with or bound to a porous membrane or a nonwoven fabric pasted to the substrate so as to close the through-holes.
- 45 4. The reaction probe chip of any one of claims 1 to 3,  
50     wherein  
50     the probe molecules are DNA's, RNA's or PNA's and fragments thereof, oligonucleotides having arbitrary base sequences, antigens, antibodies or epitopes, and enzymes, proteins or functional site polypeptide chains thereof.
- 55 5. A reaction product detection system adapted to  
55     flow a sample simultaneously and slowly through a plurality of discrete through-holes regularly arranged in a substrate, the sample including fluorescence labeled DNA to be detected, thereby

binding an analyte to probe molecules fixed in the through-holes, and detect the analyte by a fluorescence detector.

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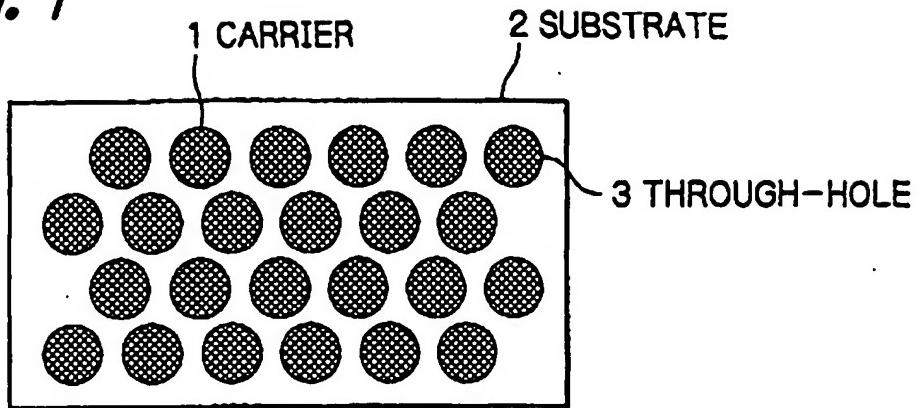
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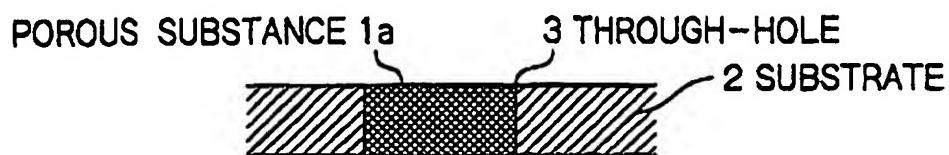
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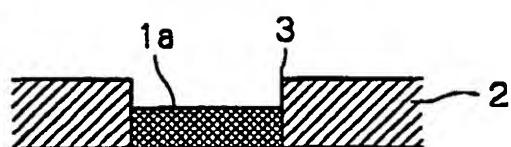
*Fig. 1*



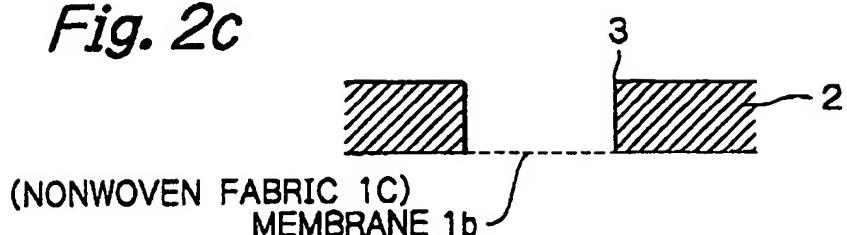
*Fig. 2a*



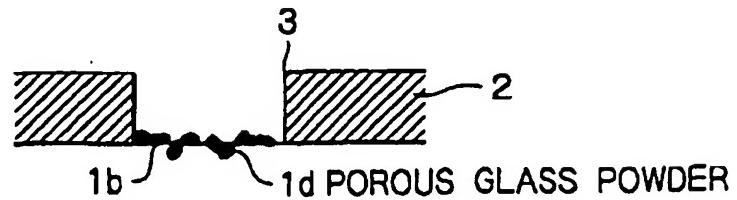
*Fig. 2b*



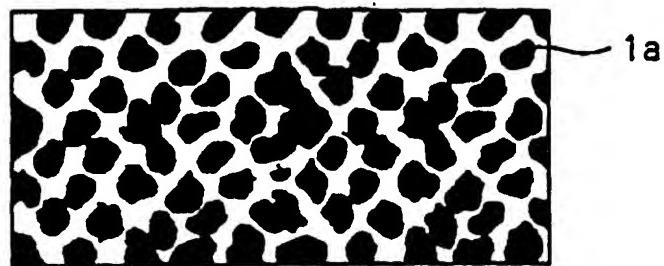
*Fig. 2c*



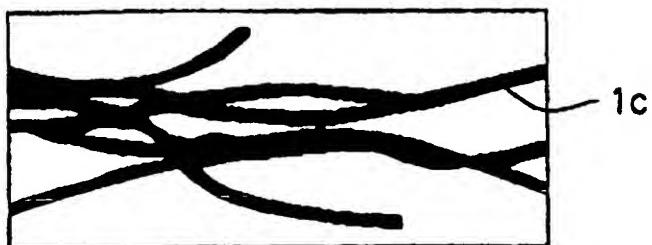
*Fig. 2d*



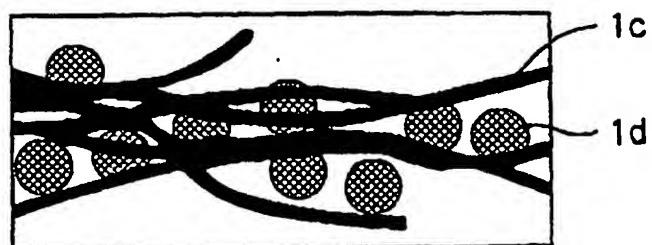
*Fig. 3e*



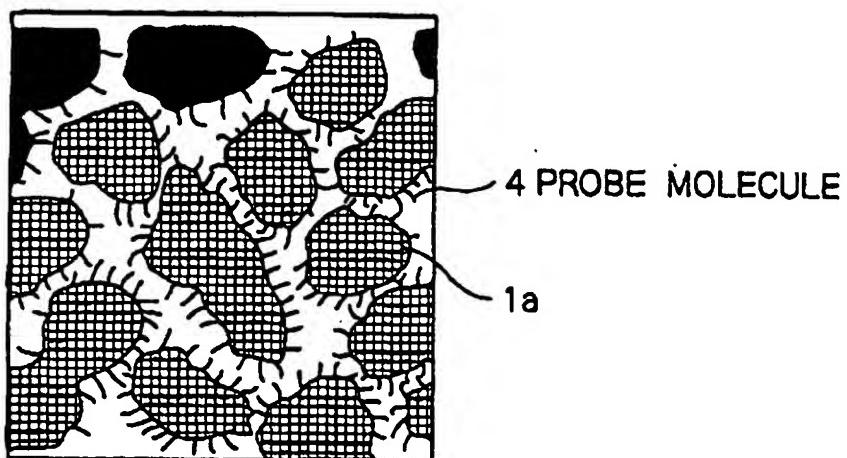
*Fig. 3f*



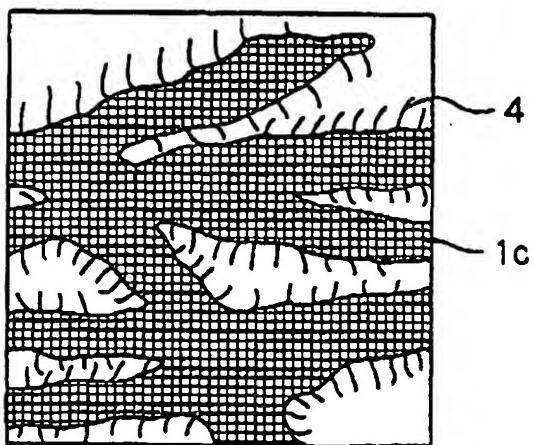
*Fig. 3g*



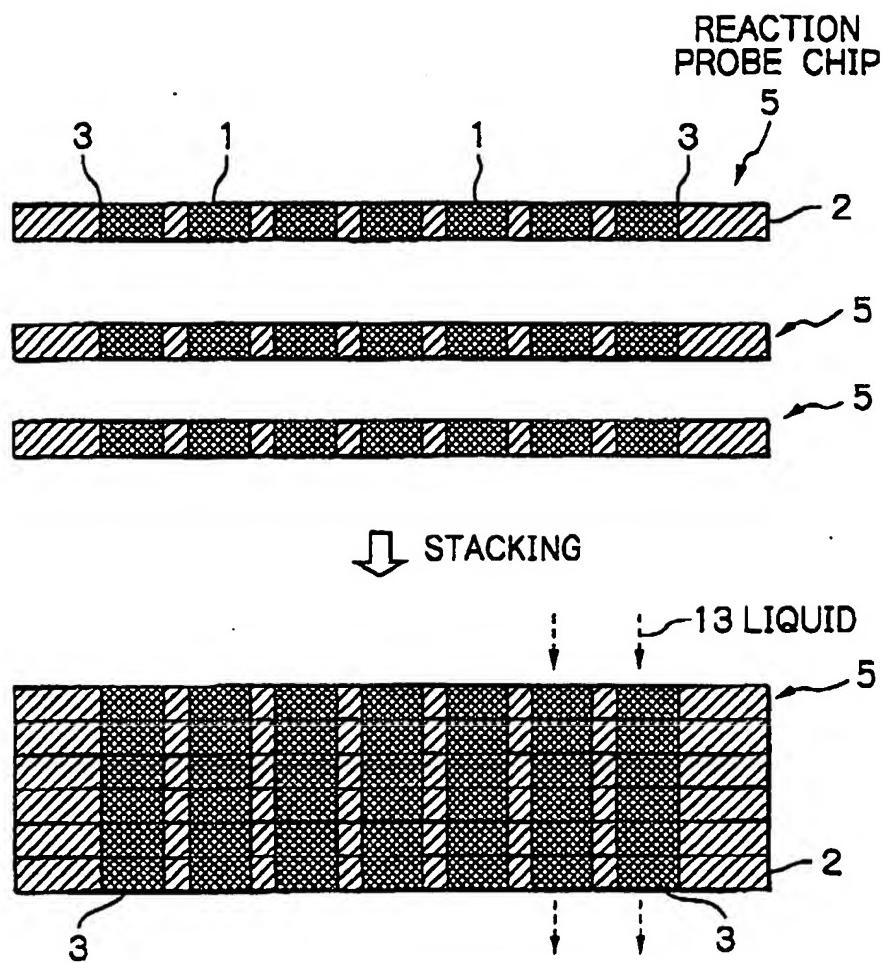
*Fig. 4h*

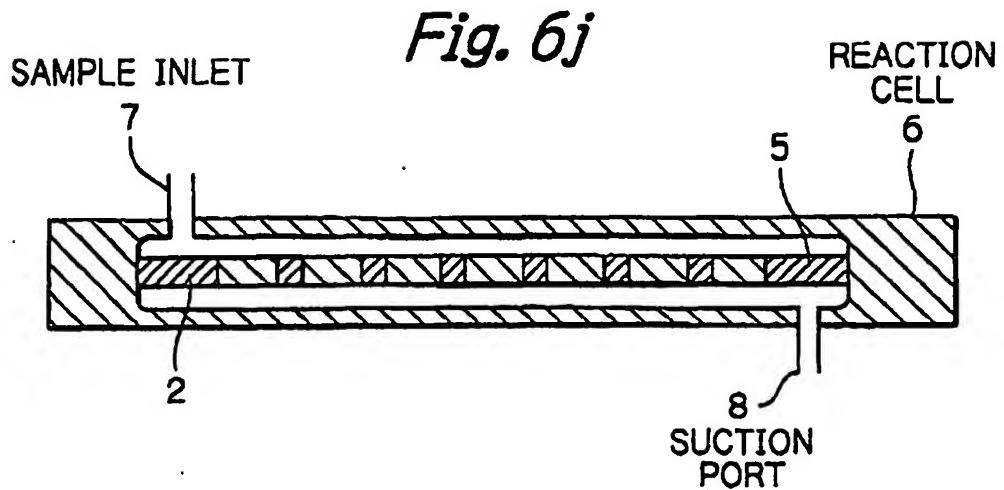


*Fig. 4i*

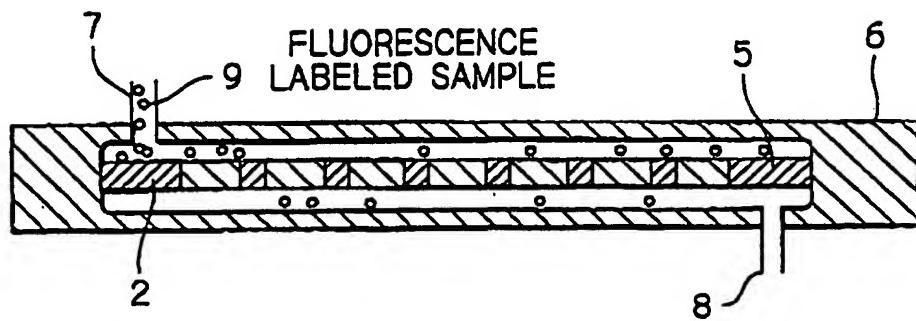


*Fig. 5*

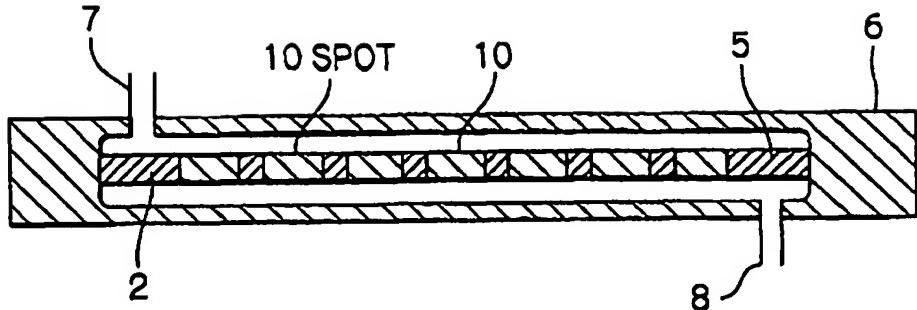




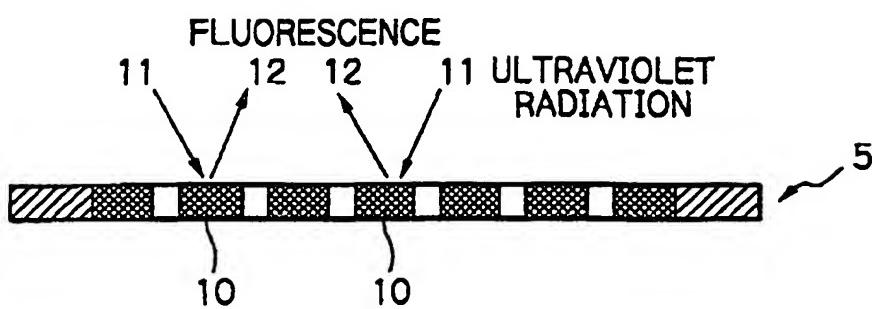
*Fig. 6k*



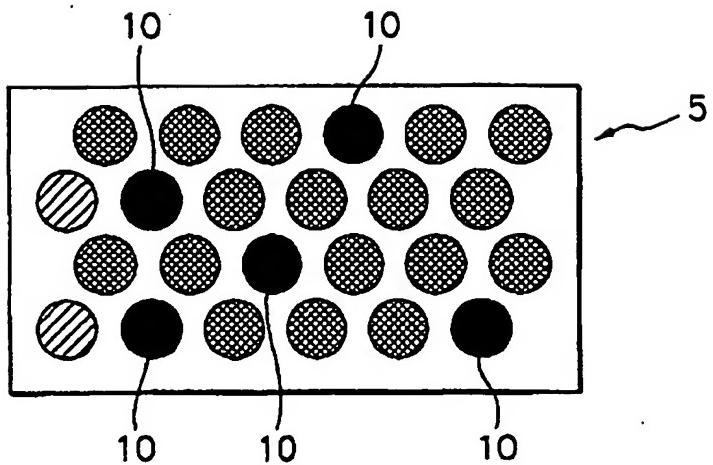
*Fig. 6l*



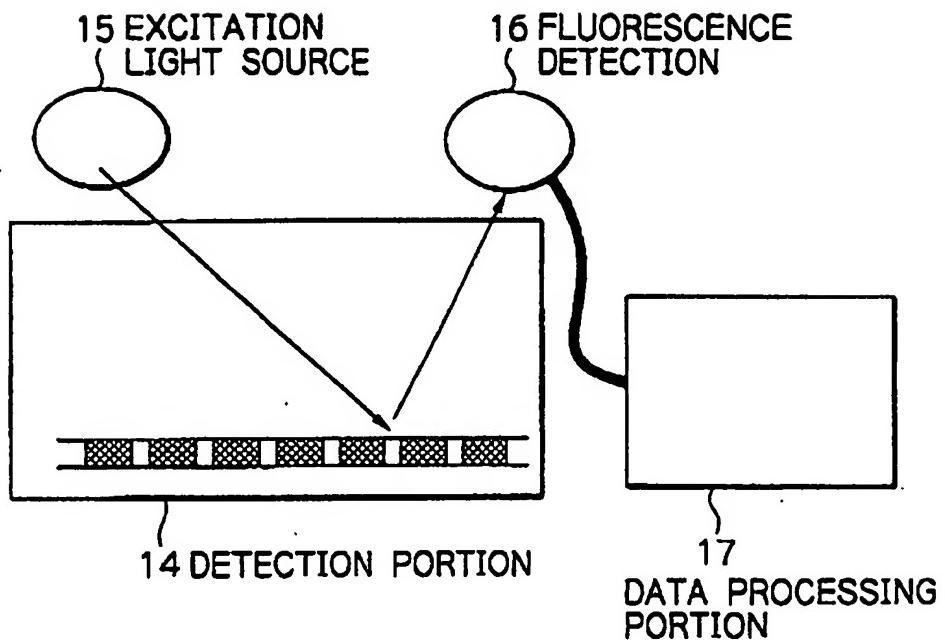
*Fig. 7*



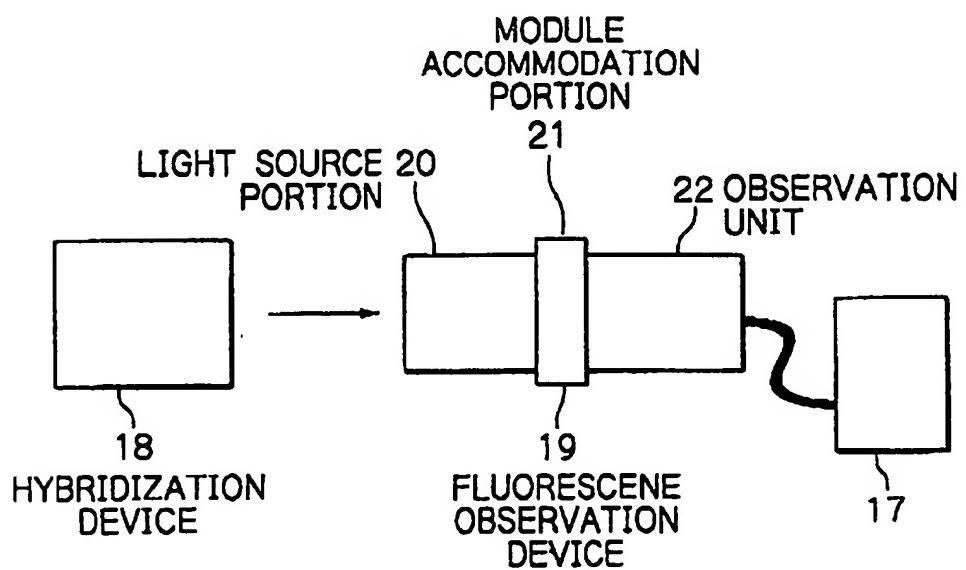
*Fig. 8*



*Fig. 9*



*Fig. 10*



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